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Effects of Using Reduced Volumes of Nonselective Enrichment Medium in Methods for the Detection of *Escherichia coli* O157:H7 from Raw Beef†

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ABSTRACT

Recent work from our laboratory revealed that tryptic soy broth (TSB) was a superior enrichment medium for use in test-and-hold *Escherichia coli* O157:H7 methods at levels down to a ratio of three volumes of medium to one volume of sample. Lower ratios were examined for their effect on results obtained from culture isolation, the BAX *E. coli* O157:H7 MP assay, and the Assurance GDS *E. coli* O157:H7 assay. Ground beef and boneless beef trim were inoculated with a high level (170 CFU/65 g of ground beef and 43 CFU/65 g of trim) and a low level (17 CFU/65 g of ground beef and 4 CFU/65 g of trim) of *E. coli* O157:H7 and enriched in 3, 1, 0.5, and 0 volumes of TSB. The volume of TSB used did not affect *E. coli* O157:H7 detection by culture isolation, Assurance GDS detection in ground beef or trim, or the BAX MP assay detection in ground beef. However, BAX MP assay detection of *E. coli* O157:H7 in beef trim was 50, 42, and 33% positive when enrichment volumes of 0.5×, 1×, and 3×, respectively, were used. Optimum results with all methods were obtained using 1 volume of TSB. We concluded that detection test results can be considered valid as long as enrichment medium is used, even when it is less than the specified 3 or 10 volumes.

Escherichia coli O157:H7 is a pathogen that has been associated with disease outbreaks involving meat, produce, and water *(7, 10, 16).* This pathogen was identified as a foodborne threat during outbreaks associated with ground beef in the 1980s and early 1990s *(14, 17).* The U.S. Department of Agriculture Food Safety and Inspection Service implemented several regulations aimed at eliminating this pathogen from red meat *(18).* At the same time, the public and private research sectors have worked to help the beef processing industry implement antimicrobial interventions that reduced *E. coli* O157:H7 contamination *(5, 9, 13).* Unfortunately, interventions have not completely eliminated *E. coli* O157:H7 on beef, and sporadic beef-associated infections continue to occur.

The beef industry responded to the safety concerns surrounding *E. coli* O157:H7 contamination in ground beef and implemented a test-and-hold process, in which a processor samples boneless beef trim (the raw material used to make ground beef) or the ground beef, enriches the sample for growth in bacterial medium, and then tests the enrichment culture for the presence of *E. coli* O157:H7 *(4, 12).* The product does not enter into commerce unless the sample contains no detectable *E. coli* O157:H7. Numerous products are available for endpoint testing of ground beef bacterial enrichment cultures, and some of those tests were recently studied by our group to determine the efficacy of these methods for detecting *E. coli* O157:H7 in ground beef *(2).*

Optimization of three testing attributes (i.e., detection time, specificity, and sensitivity) is critical to the success of test-and-hold programs. Guerini et al. *(6)* evaluated *E. coli* O157:H7 growth media for use in test-and-hold procedures and described the superiority of tryptic soy broth (TSB) as an inexpensive and effective enrichment medium. They also reported that reduced ratios of one part product (trim or ground beef) to three parts TSB, as opposed to the traditional 1:10 dilution of product to medium, improved the economy of enrichment but had no deleterious effects on detection. Thus, decreased enrichment volumes may still provide the proper nutrition and environment for *E. coli* O157:H7 to grow to detectable levels in a rapid fashion while increasing the concentration of the *E. coli* O157:H7 in the medium.

The current *E. coli* O157:H7 test-and-hold system used by the meat industry often relies on samples being shipped to third-party laboratories. In the laboratory, occasional errors can occur that may result in less than the specified amount of enrichment medium or no enrichment medium being added to a sample before incubation. Ratios of product to medium of less than 1:3 have not been evaluated. The studies presented here were conducted to compare the effects of reducing the ratio of product to medium (1:1,

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† Names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product, and the use of the name by U.S. Department of Agriculture implies no approval of the product to the exclusion of others that may also be suitable.

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1:0.5, and 1:0) on the detection of *E. coli* O157:H7 by standard culture techniques and common molecular tests.

MATERIALS AND METHODS

Study design. Samples ($n = 12$) of beef trim or ground beef (65 g) were inoculated with a low (approximately 10 CFU) or a high (approximately 100 CFU) concentration of *E. coli* O157:H7. One set of samples inoculated at each level were enriched in 3, 1, 0.5, or 0 volumes of TSB. After incubation to enrich for *E. coli* O157:H7, 1 volume of TSB was added to the 0-volume sample and homogenized. Equal volumes of each enrichment were then removed for *E. coli* O157:H7 detection by culture isolation or molecular methods: the BAX *E. coli* O157:H7 MP assay (DuPont, Wilmington, Del.) and the Assurance GDS *E. coli* O157:H7 assay (BioControl, Bellevue, Wash.). Enrichments also were directly spiral plated for assessment of background bacterial growth.

Preparation of inocula. For each day of the experiment, a cocktail of five well-characterized *E. coli* O157:H7 strains (55AC1, 114AC1, 131AC1, 237AC1, and 299AB3) *(3)* was used as the inoculum. The inoculum was prepared by growing one colony of each strain to stationary phase (36 h at 37°C) in 10 ml of TSB and then cold stressing it during dilution steps. Each stationary-phase culture was thoroughly mixed by vortexing, and then equal amounts (1 ml) were combined, mixed, and placed on ice. The starting concentration of cells was assumed to be 10^9 CFU/ml. One milliliter of the 10^9 CFU/ml cocktail was serially diluted 1:10 five times (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) in 9 ml of refrigerator-chilled buffered peptone water (BPW; approximately 10°C) in test tubes that were maintained on ice. Further serial dilutions (10^{-6}, 10^{-7}, and 10^{-8}) were scaled up to provide adequate volumes for multiple inoculations of samples. The 10^{-5} dilution was serially diluted by mixing 2 ml into 18 ml. Twelve milliliters of this solution was serially diluted two times into 108 ml of BPW to provide 108 ml of a 10^{-7} dilution and 120 ml of a 10^{-8} dilution. The 10^{-7} dilution was used as the high inoculum (presumed 100 CFU/ml), and the 10^{-8} dilution was used as the low inoculum (presumed 10 CFU/ml). The actual CFU per milliliter in each day's inoculum were determined by performing colony counts on the 10^{-5}, 10^{-6}, and 10^{-7} dilutions that had been spread plated in quadruplicate onto tryptic soy agar plates and incubated for 16 h at 37°C. The high and low inocula prepared the first day (for beef trim samples) were calculated to be 43 and 4.3 CFU/ml, respectively, and those prepared on the second day (for ground beef samples) were calculated to be 170 and 17 CFU/ml, respectively.

Inoculation and enrichment. Beef trimmings (50:50, fat:trim) and ground beef (80:20, lean:fat) that had never been frozen were obtained from a local abattoir-butcher shop. The trim and ground beef were both fresh cut and ground the day of purchase. Both products were packaged in twist-tie plastic bags and kept refrigerated until use. The beef trim was used 1 day after purchase, and the ground beef was used 2 days after purchase. The outer surfaces of the beef trimmings were cut away with a knife and collected to form a sample equivalent to that used in the "$n = 60$" method of testing beef trimmings destined for ground beef *(11)*. Sixty-five grams of trimmings and ground beef were weighed out and placed in Whirl-Pak filter bags (Nasco, Fort Atkinson, Wis.). One milliliter of either the low or the high inoculum was added to the trim or ground beef and thoroughly mixed with a Lab Blender (BagMixer 400VW, Interscience Laboratories Inc., Weymouth, Mass.) for 30 s at 420 rpm. The samples were then divided into sets of 12 high inocula and 12 low inocula and enriched by the addition of 0× (0 ml), 0.5× (32 ml), 1× (64 ml), or 3× (194 ml) TSB at room temperature. After TSB was added, each sample was again mixed in the lab blender, sealed, and incubated in a programmable incubator set for 12 h at 42°C followed by a 4°C hold until the samples were processed for *E. coli* O157:H7 detection (approximately 4 to 6 h).

Assessment of background bacterial growth. Fifty microliters of each enrichment was spiral dilution plated using a spiral plater (Autoplater 4000, Spiral Biotech, Norwood, Mass.) onto a ChromeAgar (DRG, Mountainside, N.J.) plate supplemented with 5 mg/liter novobiocin and 1 mg/liter potassium tellurite and incubated at 42°C for 16 h. After incubation, the ratio of suspect *E. coli* O157:H7 colonies (pink) to nonsuspect colonies (white and/or blue) was determined by counting the colonies of each type (suspect and nonsuspect) at the outside edges of the dilution plate. The values for background growth are given as the percentage of nonsuspect colonies in the total number of colonies counted. To ensure suspect colonies were accurately recorded, Oxoid DrySpot O157 latex agglutination tests (Thermo Fisher Scientific, Waltham, Mass.) were used to confirm the suspect phenotype.

Culture detection. One milliliter of each enrichment was removed from the sample bag for culture isolation of *E. coli* O157:H7 by immunomagnetic separation (IMS) as previously described *(1)*.

Molecular detection. Sample enrichments were tested for the presence of *E. coli* O157:H7 using (i) the commercial BAX *E. coli* O157:H7 MP kit with BAX lysis buffer and BAX instruments according to the manufacturer's instructions, (ii) the commercial Assurance GDS *E. coli* O157:H7 kit and the prescribed IMS followed by DNA detection of target in a BioControl GDS instrument, and (iii) a multiplex PCR assay for specific *E. coli* O157:H7 gene markers *(8)*. The multiplex PCR assay was performed using 1 μl of direct sample enrichment in a 25-μl PCR mix and using 20 μl of BAX lysis buffer in a 25-μl PCR mix. Multiplex PCR products were amplified in a MJR PTC 100 thermocycler (Bio-Rad Laboratories, Hercules, Calif.) according to previously described parameters *(1)* and resolved on 2.5% agarose gels stained with ethidium bromide. Multiplex reactions were considered positive for *E. coli* O157:H7 only when both the rfb^{O157} and $fliC^{H7}$ products were present with one additional product (stx_1, stx_2, or *eae*) and were considered negative only when no PCR products were present. When PCR products were present but did not fit the definition of a positive sample, the sample was considered indeterminable.

Statistics. Comparisons of frequencies of *E. coli* O157:H7 detection in each set of samples were made using PROC FREQ and Mantel-Haenszel chi-square analysis in SAS (SAS Institute, Cary, N.C.).

RESULTS AND DISCUSSION

The volume of enrichment medium added to beef trim and ground beef did not affect the culture isolation of *E. coli* O157:H7 from the samples (Table 1); however, significant differences in the levels of background bacterial growth that could interfere with culture isolation were found (Table 2). All but three samples of trim and ground beef contained *E. coli* O157:H7 as determined by culture isolation. Two culture-negative trim samples had been inoculated with low numbers and enriched without medium. The third was a low-inoculum ground beef sample enriched

TABLE 1. *Effects of reduced enrichment volume on the culture isolation of* E. coli *O157:H7 from beef trim and ground beef*[a]

Sample type	No. positive/no. tested (%) at medium-to-sample ratios of[b]: 0×[c]	0.5×	1×	3×
Trim				
4.3 CFU/65 g	10/12 A (83)	12/12 A (100)	12/12 A (100)	12/12 A (100)
43 CFU/65 g	12/12 A (100)	12/12 A (100)	12/12 A (100)	12/12 A (100)
Ground beef				
17 CFU/65 g	12/12 A (100)	12/12 A (100)	12/12 A (100)	11/12 A (92)
170 CFU/65 g	12/12 A (100)	12/12 A (100)	12/12 A (100)	12/12 A (100)
All combined	46/48 A (96)	48/48 A (100)	48/48 A (100)	47/48 A (98)

[a] Culture isolation was performed by immunomagnetic separation and plating to selective agar. Values represent the number of culture-positive samples/the total number of samples tested (percentage of positive samples). Within a row, values with common letters are not significantly different ($P > 0.05$).

[b] Sixty-five grams of sample was enriched in 0 ml (0×), 32 ml (0.5×), 64 ml (1×), or 194 ml (3×) of TSB.

[c] The 0× samples were homogenized with 1 volume (64 ml) of TSB after enrichment incubation to provide a 1-ml volume for culture isolation.

with 3 volumes of medium. The two culture-negative trim samples were considered negative because these samples also produced negative results with the other detection methods. These two samples probably received no *E. coli* O157:H7 cells from the inoculum because the cells at this level (4.3 CFU/ml) were not evenly distributed throughout the tube holding the inoculum. Therefore, for the of trim inoculated with low numbers, only the 10 samples determined to contain *E. coli* O157:H7 were used for data analysis and interpretation. Concerning the ground beef sample that was culture negative, it is unlikely that uneven distribution of cells in the low inoculum resulted in a 1-ml aliquot that had no cells because this inoculum was four-fold higher in concentration than that used for the trim. During the IMS steps of culture isolation, bead recovery was variable and was poorer in samples that had a higher fat content. The ground beef sample in question was one in which poor bead recovery was observed, which could explain why the culture isolation did not produce a positive result. This negative ground beef sample produced a positive result when the other methods were used. Therefore, it was included in subsequent data analyses.

TABLE 2. *Effects of reduced enrichment volume on background growth compared with suspect* E. coli *O157:H7 growth in enrichments of inoculated beef samples*[a]

Sample type	% of nonsuspect *E. coli* O157:H7 colonies in cultures with medium-to-sample ratios of[b]: 0×[c]	0.5×	1×	3×
Trim				
4.3 CFU/65 g	25 B	99 A	95 A	99 A
43 CFU/65 g	20 C	60 B	65 AB	85 A
Ground beef				
17 CFU/65 g	70 A	40 B	40 B	10 C
170 CFU/65 g	40 B	60 A	40 B	1 C

[a] Background growth in enrichments was determined by enumerating suspect *E. coli* O157:H7 colonies (pink) and nonsuspect colonies (white and/or blue) on spiral dilution plates. Values are the proportion of colonies determined to be background growth, i.e., the percentage of nonsuspect colonies in the total number of colonies counted. Within a row, values with common letters are not significantly different ($P > 0.05$).

[b] Sixty-five grams of sample was enriched in 0 ml (0×), 32 ml (0.5×), 64 ml (1×), or 194 ml (3×) of TSB.

[c] The 0× samples were homogenized with 1 volume (64 ml) of TSB after enrichment incubation to provide sufficient volume for background determination.

The inoculum used in these assays was a cocktail of five strains of *E. coli* O157:H7. These five strains have previously been used individually at levels as low as 1.7 CFU per sample in evaluations of culture and molecular methods *(2).* In the earlier evaluations, each strain grew to detectable levels, but the levels of competing background bacteria were unknown. In the experiments presented here, we evaluated the amount of background bacterial growth in each enrichment by enumerating the outer regions of a spiral dilution plate. Two opposite effects were observed (Table 2). In beef trim, the ratio of background growth to *E. coli* O157:H7 growth increased with additions of larger volumes of medium, whereas in ground beef enrichments marked decreases in background growth occurred with additions of larger volumes of medium. Generally, samples inoculated at low levels had the highest percentage of background bacterial colonies, whereas samples inoculated at high levels had lower percentages of background colonies. These findings for background bacterial growth in ground beef fit with the fact that we used a 12-h 42°C incubation protocol to select for *E. coli* O157:H7. Under these conditions, it is expected that given enough medium the *E. coli* O157:H7 would be able to outgrow the competing bacteria in the ground beef. However, the contrasting finding in trim is more difficult to explain. Portions of this same 50% fat trim were used to prepare the ground beef used in our studies. The bacteria in the trim were likely diluted out during blending and grinding with lean trim. Therefore, the types of bacteria that made up the background flora in the trim and/or the fact that these bacteria were more concentrated

TABLE 3. *Effects of reduced enrichment volume on the detection of* E. coli *O157:H7 from beef trim and ground beef using the BioControl Assurance GDS* E. coli *O157:H7 assay*[a]

Sample type	No. positive/no. tested (%) at medium-to-sample ratios of[b]:			
	0×[c]	0.5×	1×	3×
Trim				
4.3 CFU/65 g	9/10 A (90)	12/12 A (100)	12/12 A (100)	8/12 A (67)
43 CFU/65 g	12/12 A (100)	12/12 A (100)	12/12 A (100)	12/12 A (100)
Ground beef				
17 CFU/65 g	12/12 A (100)	12/12 A (100)	12/12 A (100)	12/12 A (100)
170 CFU/65 g	12/12 A (100)	12/12 A (100)	12/12 A (100)	12/12 A (100)
All combined	45/46 A (98)	48/48 A (100)	48/48 A (100)	44/48 A (92)

[a] Values represent the number of Assurance GDS *E. coli* O157:H7–positive samples/the total number of samples tested (percentage of positive samples). Within a row, values with common letters are not significantly different ($P > 0.05$).

[b] Sixty-five grams of sample was enriched in 0 ml (0×), 32 ml (0.5×), 64 ml (1×), or 194 ml (3×) of TSB.

[c] The 0× samples were homogenized with 1 volume (64 ml) of TSB after enrichment incubation to provide a 1-ml volume for GDS detection.

in the trim may be the explanation for these contrary results.

The amount of culture enrichment medium used did not affect the detection of *E. coli* O157:H7 in the ground beef and trim when the Assurance GDS was used (Table 3). The detection of *E. coli* O157:H7 in ground beef with BAX MP assay also was not affected by the different enrichment volumes (Table 4). However, detection in beef trim was more problematic. With the BAX MP assay, only 50% or less of the positive trim samples inoculated at the low level were detected, whereas with the GDS two-thirds or more of these samples were detected. When enrichment medium was left out of the beef trim samples, only 40 and 42% of low and high-inoculum samples, respectively, were detected with the BAX MP assay. When medium-to-sample ratios of 0.5, 1, and 3 were used in the trim inoculated at low levels, only 50, 42, and 33% of *E. coli* O157:H7, respectively, were detected with the BAX MP assay. The reason that the Assurance GDS was more successful than the BAX MP assay for detecting *E. coli* O157:H7 in samples enriched with decreased volumes of medium or low inoculation levels probably is associated with the IMS step in the GDS, which occurs before gene amplification. One milliliter was used for IMS in the Assurance GDS, whereas 5 μl was used in the BAX lysis step that precedes the detection step. The Assurance GDS concentrates *E. coli* O157:H7 by IMS to detectable levels that could be missed in the BAX MP assay.

The multiplex PCR assay used in our laboratory *(8)* was similarly affected by the absence of enrichment medium in trim samples but at both levels of inoculation. In our laboratory, this multiplex PCR assay typically is used to confirm a suspect isolate as *E. coli* O157:H7 rather than as a method to screen for *E. coli* O157:H7 in sample enrichments. We used this multiplex PCR assay to answer the question of whether *E. coli* O157:H7 could be detected in sample enrichment cultures by using 2 μl of the enrichment culture directly in the PCR mix instead of using a DNA preparation made in BAX lysis buffer. Direct testing of the enrichments resulted in no PCR products, probably because

TABLE 4. *Effects of reduced enrichment volume on the detection of* E. coli *O157:H7 from beef trim and ground beef using the DuPont BAX* E. coli *O157:H7-MP assay*[a]

Sample type	No. positive/no. tested (%) at medium-to-sample ratios of[b]:			
	0×[c]	0.5×	1×	3×
Trim				
4.3 CFU/65 g	4/10 A (40)	6/12 A (50)	5/12 A (42)	4/12 A (33)
43 CFU/65 g	5/12 B (42)	11/12 A (92)	12/12 A (100)	12/12 A (100)
Ground beef				
17 CFU/65 g	11/12 A (92)	11/12 A (92)	12/12 A (100)	11/12 A (92)
170 CFU/65 g	12/12 A (100)	11/12 A (92)	12/12 A (100)	12/12 A (100)
All combined	32/46 A (70)	39/48 A (81)	41/48 A (85)	39/48 A (81)

[a] Values represent the number of BAX *E. coli* O157:H7 MP assay–positive samples/the total number of samples tested (percentage of positive samples). Within a row, values with common letters are not significantly different ($P > 0.05$).

[b] Sixty-five grams of sample was enriched in 0 ml (0×), 32 ml (0.5×), 64 ml (1×), or 194 ml (3×) of TSB.

[c] The 0× samples were homogenized with 1 volume (64 ml) of TSB after enrichment incubation to provide sufficient volume for BAX MP detection.

of various factors such as excessive DNA and cellular debris, possible inhibitors in the enrichment, and the effects of fat in the enrichment. Reactions that were performed with the BAX lysis buffer did work well in the multiplex system, nearly as well as did the BAX MP assay. Seventy-eight percent of the samples detected with the multiplex PCR assay met the criteria for positive interpretation compared with 79% with the BAX MP assay and 97% with the Assurance GDS (data not shown).

The correlation between culture and molecular results was very high. The samples that were negative by culture also were negative by both the BAX MP assay and the GDS. All samples that were considered negative with the GDS also were negative with the BAX assay but not vice versa. The ratio of *E. coli* O157:H7 growth to background bacterial growth probably played a part in the observed results of the commercial detection systems. Samples that were negative with the BAX MP assay but positive after culture and with the Assurance GDS had a much lower concentration of *E. coli* O157:H7 in the enrichments. Because the BAX MP assay is more sensitive to the concentration of *E. coli* O157:H7 than are methods that include IMS, enrichments with low observed levels of *E. coli* O157:H7 probably were not detected.

Both the BAX MP assay and the Assurance GDS specify media other than TSB in their protocols and shorter enrichment times than used in the present study. We used TSB so results could be compared with those of previous studies. If the experiments presented here were repeated using either the BAX MP medium or the GDS mEHEC medium, different results may have been obtained. Both of these media are selective for *E. coli* O157:H7 and have additional proprietary ingredients that reduce background bacteria. Despite the selective nature of these media, our group has previously found that TSB is no less effective an enrichment medium *(7),* and since that report TSB has become an economical and accepted replacement for these media in test-and-hold programs.

The results obtained with the BAX MP assay and the GDS for trim samples inoculated with low numbers of cells indicated what may be a beneficial effect of using lower volumes of enrichment medium. The *E. coli* O157:H7 cells were concentrated in the reduced volumes so that when 0.5×, 1×, and 3× medium volumes were used, the detection prevalence was 50, 42, and 33% with the BAX MP assay and 100, 100, and 67% with the GDS.

The *E. coli* O157:H7 cells used in our experiments were laboratory grown to stationary phase and cold stressed during dilution steps before use. They were not additionally stressed by acid, heat, or freezing as would occur during beef processing. Therefore, our inoculated strains may not perform like actual process-surviving *E. coli* O157:H7. The methods of inoculum preparation used here are similar to those used previously to determine media efficacy *(6)* and to evaluate differences in detection tests *(2).*

These studies were conducted to determine the effects of using reduced volumes of TSB for enrichment and detection of *E. coli* O157:H7 inoculated into ground beef and beef trim. Volumes as low as half the sample size can be used with no effect on *E. coli* detection with the commercial molecular systems, but no enrichment medium was required for *E. coli* detection by culture isolation techniques. Optimum results were observed using as little as 1 volume of TSB per sample. Therefore, when less than the specified 3 or 10 volumes of medium are added to an *E. coli* O157:H7 detection test, the result will not necessarily be erroneous. Because inoculated samples were negative for *E. coli* O157:H7 in some of the experimental situations, we recommend that other rapid tests also be evaluated for such realistic variations in enrichment medium.

Note added in proof. Since the completion of these studies, we have become aware of a patent filed that proposes enrichment methods for the detection of pathogens and other microbes in food, water, etc., at ratios of sample-to-medium volumes of 1:0, 1:0.1, and 1:2 (wt/vol) or greater *(15).*

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REFERENCES

1. Arthur, T. M., J. M. Bosilevac, D. M. Brichta-Harhay, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2007. Effects of a minimal hide wash cabinet on the levels and prevalence of *Escherichia coli* O157:H7 and *Salmonella* on the hides of beef cattle at slaughter. *J. Food Prot.* 70:1076–1079.
2. Arthur, T. M., J. M. Bosilevac, X. Nou, and M. Koohmaraie. 2005. Evaluation of culture- and PCR-based detection methods for *Escherichia coli* O157 in ground beef. *J. Food Prot.* 68:1566–1574.
3. Barkocy-Gallagher, G. A., T. M. Arthur, G. R. Siragusa, J. E. Keen, R. O. Elder, W. W. Laegreid, and M. Koohmaraie. 2001. Genotypic analyses of *Escherichia coli* O157:H7 and O157 nonmotile isolates recovered from beef cattle and carcasses at processing plants in the Midwestern states of the United States. *Appl. Environ. Microbiol.* 67:3810–3818.
4. Brabban, A. D., D. A. Nelsen, E. Kutter, T. S. Edrington, and T. R. Callaway. 2004. Approaches to controlling *Escherichia coli* O157:H7, a foodborne pathogen and an emerging environmental hazard. *Environ. Rev.* 6:208–229.
5. Castillo, A., L. M. Lucia, K. J. Goodson, J. W. Savell, and G. R. Acuff. 1999. Decontamination of beef carcass surface tissue by steam vacuuming alone and combined with hot water and lactic acid sprays. *J. Food Prot.* 62:146–151.
6. Guerini, M. N., T. M. Arthur, S. D. Shackelford, and M. Koohmaraie. 2006. Evaluation of *Escherichia coli* O157:H7 growth media for use in test-and-hold procedures for ground beef processing. *J. Food Prot.* 9:1007–1011.
7. Hrudey, S. E., P. Payment, P. M. Huck, R. W. Gillham, and E. J. Hrudey. 2003. A fatal waterborne disease epidemic in Walkerton, Ontario: comparison with other waterborne outbreaks in the developed world. *Water Sci. Technol.* 47:7–14.
8. Hu, Y., Q. Zhang, and J. C. Meitzler. 1999. Rapid and sensitive detection of *Escherichia coli* O157:H7 in bovine faeces by multiplex PCR. *J. Appl. Microbiol.* 87:867–876.
9. Kang, D. H., M. Koohmaraie, and G. R. Siragusa. 2001. Application of multiple antimicrobial interventions for microbial decontamination of commercial beef trim. *J. Food Prot.* 64:168–171.
10. Kassenborg, H. D., C. W. Hedberg, M. Hoekstra, M. C. Evans, A. E. Chin, R. Marcus, D. J. Vugia, K. Smith, S. D. Ahuja, L. Slutsker, P. M. Griffin, and the Emerging Infections Program FoodNet Working Group. 2004. Farm visits and undercooked hamburgers as major risk factors for sporadic *Escherichia coli* O157:H7 infection: data

from a case-control study in 5 FoodNet sites. *Clin. Infect. Dis.* 38(Suppl. 3):S271–S278.
11. Kiermeier, A., G. Holds, M. Lorimer, I. Jensen, and J. Sumner. 2007. Sampling cartons of beef trim for microbiological analysis: comparison of portions versus surface slices. *Food Prot. Trends* 27:899–902.
12. Koohmaraie, M., T. M. Arthur, J. M. Bosilevac, M. Guerini, S. D. Shackelford, and T. L. Wheeler. 2005. Post-harvest interventions to reduce/eliminate pathogens in beef. *Meat Sci.* 71:79–91.
13. Phebus, R. K., A. L. Nutsch, D. E. Schafer, R. C. Wilson, M. J. Riemann, J. D. Leising, C. L. Kastner, J. R. Wolf, and R. K. Prasai. 1997. Comparison of steam pasteurization and other methods for reduction of pathogens on surfaces of freshly slaughtered beef. *J. Food Prot.* 60:476–484.
14. Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Hebert, E. S. Olcott, L. M. Johnson, N. T. Hargrett, P. A. Blake, and M. L. Cohen. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N. Engl. J. Med.* 308:681–685.
15. Samadpour, M. 2004. U.S. patent application 20040241644.
16. Sivapalasingam, S., C. R. Friedman, L. Cohen, and R. V. Tauxe. 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *J. Food Prot.* 67: 2342–2353.
17. Tuttle, J., T. Gomez, M. P. Doyle, J. G. Wells, T. Zhao, R. V. Tauxe, and P. M. Griffin. 1999. Lessons from a large outbreak of *Escherichia coli* O157:H7 infections: insights into the infectious dose and method of widespread contamination of hamburger patties. *Epidemiol. Infect.* 122:185–192.
18. U.S. Department of Agriculture, Food Safety and Inspection Service. 1996. Pathogen reduction; hazard analysis and critical control point (HACCP) systems; final rule. *Fed. Regist.* 61:38805–38989.